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One promising new treatment modality for breast cancer is the application of vector-mediated gene therapy. A noted problem with many vector systems however, including both viral and non-viral vectors, used for gene therapy is the lack of efficient and targeted delivery to the primary tumor and disseminated metastases. To address this issue, we propose the use of $CD34^+$ and/or $Flk\text{-}1^+$ endothelial progenitor cells (EPCs), which have the propensity of homing to sites of neovascularization. Key to the success of this approach is efficient genetic modification of the EPCs. In this regard, we have shown previously that $CD34^+$ EPCs are efficiently transduced using live-viral vectors with relatively low doses and associated toxicity. We hypothesize that the modified EPCs can, after intravascular injection, localize into sites of tumor neovascularization and deliver therapeutic payloads. Further, the natural targeting capacity of EPCs will allow their use vectors for gene therapy of both local and disseminated breast cancer.

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Introduction

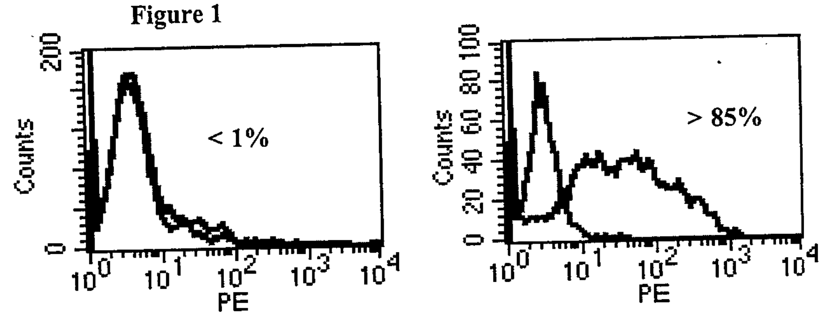
The development of resistance to radiation and chemotherapeutic agents that cause DNA damage is a major problem for the treatment of breast cancer (*1*), which argues for the development of new therapeutic agents that can either augment the effects of radiation and chemotherapy or that can be applied as an adjunct or alternative treatments. One promising new treatment modality is the application of vector-mediated gene therapy. A noted problem with many vectors, including both viral and non-viral vectors, used for gene therapy is the lack of efficient and targeted delivery to the primary tumor and disseminated metastases. To address this issue, we propose the use of $CD34^+$ and/or Flk-1^+ endothelial progenitor cells (EPCs), which have the propensity of homing to sites of neoangiogenesis. Key to the success of this approach is a vector system for the efficient genetic modification of the EPCs. In this regard, we have shown previously that $CD34^+$ EPCs are efficiently transduced using live-viral vector with relatively low doses and associated toxicity. One promising new treatment modality for breast cancer is the application of vector-mediated gene therapy. A noted problem with many vector systems however, including both viral and non-viral vectors, used for gene therapy is the lack of efficient and targeted delivery to the primary tumor and disseminated metastases. To address this issue, we propose the use of $CD34^+$ and/or Flk-1^+ endothelial progenitor cells (EPCs), which have the propensity of homing to sites of neovascularization. Key to the success of this approach is the efficient genetic modification of the EPCs. In this regard, we have shown previously that $CD34^+$ EPCs are efficiently transduced using live-viral vectors with relatively low doses and associated toxicity. We hypothesize that the modified EPCs can, after intravascular injection, localize into sites of tumor neovascularization and deliver therapeutic payloads. To test this hypothesis, we proposed to demonstrate that the EPCs (1) localize to sites of neovascularization in a murine model, (2) express virally-encoded transgenes and (3) effect therapeutic activity at tumor engraftment sites. Importantly, the natural targeting capacity of EPCs will allow their use vectors for gene therapy of both local and disseminated disease and establish a new paradigm for the treatment of breast cancer.

Body

On 9/14/00 the Principle Investigator responsibilities for this project were reassigned to Dr. Jerry L. Blackwell. I maintained an interest during the early developmental phases of the project (*2, 3*) and enthusiastically endorsed taking over the project when Dr. Jesus Gomez-Navarro left the University of Alabama and could no longer service the grant. Since taking over the Principle Investigator responsibilities I have assigned a highly experienced research assistant, Dr. Hui LI, to work on this project. Dr. Hui currently has ~50% effort dedicated to this project. In addition, Dr. Yosuke Kawakami, a talented post doctoral fellow working in the Principle Investgator's lab, has also joined the project. Dr. Kawakami's primary role is to evaluate the *in vivo* translational components of the project.

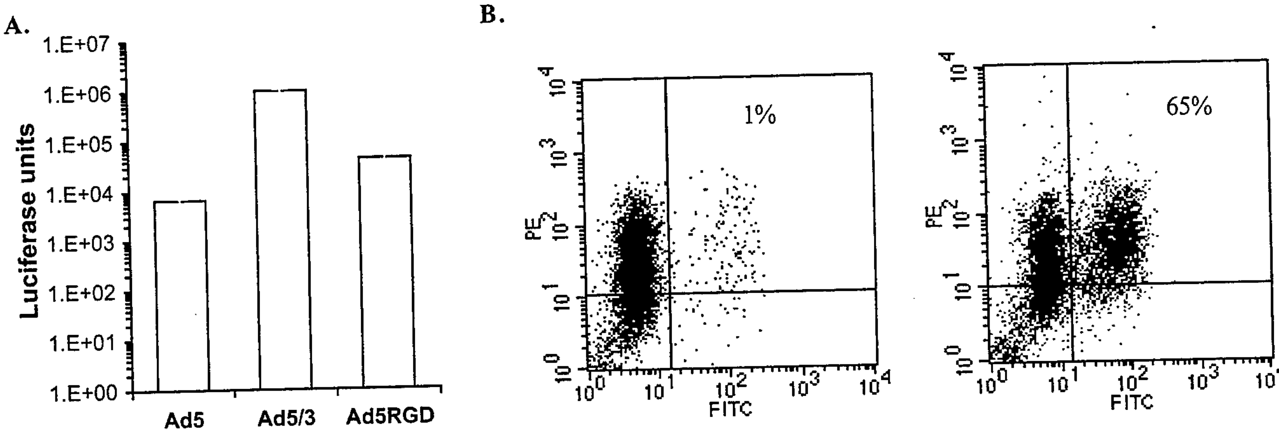
As discussed in the 2001 Annual Report, the first objectives were to develop protocols for the large-scale production of herpesvirus and purification of blood-derived CD34+ EPCs, both of which have been successfully accomplished. Figure 1 shows the purity of CD34+ EPCs before (left) and after (right) immunomagnetic positive selection from peripheral blood mononuclear cells (PBMC). Before purification < 1% of the cells are CD34-positive, yet after purification > 85% of the cells are CD34-positive. The ability to obtain a highly enriched CD34+ EPC population from a readily available blood source has been a significant accomplishment in the progression of this project.

Figure 1



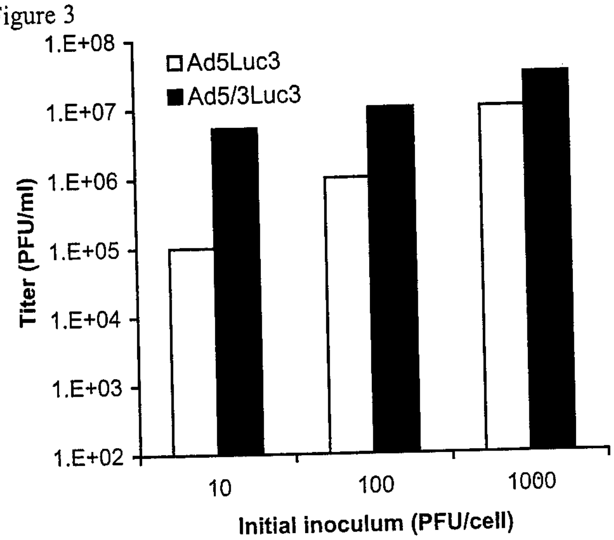
In addition to evaluating herpesvirus as a gene transfer vector for CD34+ EPCs, we have investigated the utility of adenovirus (Ad) vectors. Although we have observed that Ad serotype 5 (Ad5) is relatively inefficient at transducing CD34+ EPCs, we have shown recently that two tropism-modified Ad vectors selectively transduce these cells with high efficiency (Figure 2A). An Ad5 vector with Ad serotype 3 tropism (Ad5/3) transduced the CD34+ EPCs >2 log factors better than Ad5. An Ad5 vector with integrin tropism (Ad5RGD) infected the CD34+ EPCS ~1 log factor better than Ad5. As shown in the upper-right quadrants in Figure 2B, approximately 1% of the CD34+ EPCs are infected by Ad5 (left) compared to 65% by Ad5/3 (right).

Figure 2



Ad vectors have been extensively studied for gene therapy applications. Some of the noted advantages over other live-viral vectors include (i) the ability to easily grow and purify high titered Ad vector preparations, (ii) the Ad vector transduces both dividing and nondividing cells, and (iii) that compared to many other viral and most nonviral vectors, Ad vectors transduce a wide range of cell types. Considering these advantages and the results shown in Figure 2, we decided to further investigate the use of tropism-modified Ad vectors for gene transfer to CD34+ EPCs. In this regard, one extremely promising area in gene therapy research is the use of oncolytic Ad vectors that selectively replicate in tumor tissues. These conditionally-replicative Ad (CRAd) vectors induce oncolysis in tumor tissue, but spare normal tissues (*4*). We therefore sought to "load" CD34+ EPCs with tropism-modified CRAd vectors. The hypothesis is that the CD34+ EPCs will deliver their oncolytic payload to the tumor tissues where the CRAd would replicate. For this approach to succeed, it is necessary that the CD34+ EPCs support Ad replication, which has not been reported to date. To investigate this question, CD34+ EPCs were infected with replication-competent Ad5 or Ad5/3 vectors. Forty-eight hours later, the cells were harvested and amounts of *de novo* Ad vector production was measured by plaque assays on HEK293 cells (Figure 3).

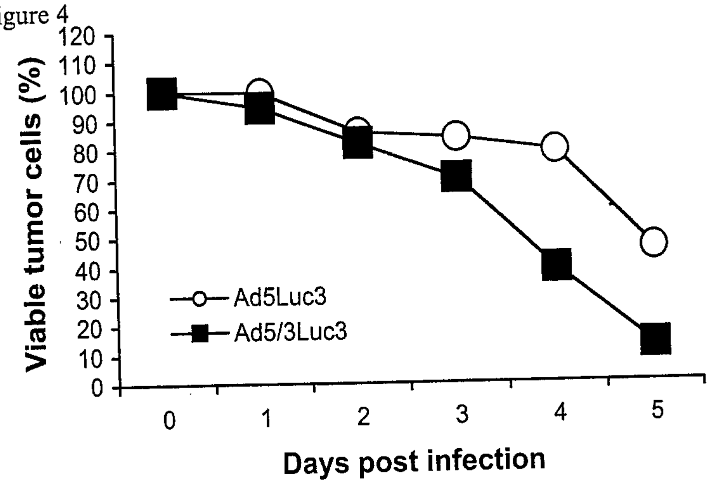
Figure 3



Infection by both Ad5 and Ad5/3 vectors resulted in *de novo* virus production, however the Ad5/3 infection resulted in much higher amounts of virus production. Importantly, these results demonstrate that CD34+ EPCs support Ad replication.

Based on the previous results, we next investigated whether CD34+ EPCs loaded with oncolytic Ad vectors could be used therapeutically to kill tumor cells in an *in vitro* cell migration model. CD34+ EPCs were infected with either replication-competent Ad5 or Ad5/3 vectors. Uninfected or infected CD34+ EPCs were then placed in the top well of a chemotaxis chamber. Breast cancer cells were placed in the bottom well of the chemotaxis

Figure 4



chamber. In addition, vascular endothelial growth factor (VEGF) was supplemented to the media in the bottom well of the chemotaxis chamber. VEGF is a known chemoattractant for CD34+ EPCs (*5, 6*). The CD34+ EPCs were allowed to migrate to the bottom chamber for 12 h, after which the top well of the chemotaxis chamber was removed. The number of tumor cells killed by the release of oncolytic Ad vectors relative to the uninfected control was determined for the next 5 days (Figure 4). The breast cancer tumor cells were efficiently killed in both groups, with the Ad5/3-infected CD34+ EPCs being more effective. These results demonstrate that CD34+ EPCs infected with replication-competent Ad vectors are able to migrate to tumor cells in response to an angiogenic stimuli and, subsequently, deliver their oncolytic payload to induce an anti-tumor effect.

We are currently seeking to establish that CD34+ EPCs can engraft into human tumor xenografts in a murine model. To establish this point, breast cancer cells were implanted subcutaneously into the flank regions of nude mice. Approximately 10 days later, CD34+ EPCs are injected intravenously via the tail vein. Twenty-one days later, VEGF receptor-2 (VEGFR-2) and angiopoictin-2 (ANG-2) mRNA expression in the breast tumor xenografts is analyzed by nested RT-PCR. Breast tumor xenografts in the mice that received the CD34+ EPCs, but not the xenografts in mice that did not receive the CD34+ EPCs, demonstrated VEGFR-2 and ANG-2 expression (data not shown). These results strongly suggest that the CD34+ EPCs migrated and engrafted into the human breast cancer xenografts. This experiment is currently being repeated with a larger number of mice in each group to confirm the first findings and to optimize the engraftment time course. Subsequently, we will pre-load the CD34+ EPCs with reporter gene-expressing (i.e. luciferase) and oncolytic Ad vectors. We also plan to genetically load the CD34+ EPCs with an imaging gene (i.e. somatostatin), which will provide a unique non-invasive method for detecting both local and disseminated tumors.

Key Research Accomplishments

- Validated protocol development for obtaining a highly enriched CD34^{+} EPCs preparation from human peripheral blood mononuclear cells
- Established another live-viral vector system for genetic-modification of CD34+ EPCs that has important translational implications
- Demonstrated that tropism-modified Ad vectors can efficiently infect and replicate in CD34+ EPCs with low toxicity
- Demonstrated that CD34+ EPCs loaded with therapeutic agents can efficiently kill cancer tumor cells
- Shown that the genetic loading process does not interfere with CD34+ EPC migration functions in an *in vitro* chemotaxis model
- Generated preliminary data suggesting that CD34+ EPCs are capable of migrating and engrafting into human breast cancer xenografts in an *in vivo* murine model

Reportable Outcomes

- Invited to present the findings of the current study as an oral presentation at the 2002 American Society of Gene Therapy meeting in Boston MA. The title of the presentation was "CD34+ Endothelial Progenitors as Cellular Vehicles for Oncolytic Adenovirus Anti-Tumor Therapy".

- Invited to present the findings of the current study as an oral presentation at the 2002 Era of Hope DOD Breast Cancer Research Program's Meeting in Orlando FL. The title of the presentation is "CD34+ Endothelial Progenitors as Cellular Vehicles for Gene Therapy of Breast Cancer".

- Manuscript in preparation entitled "Endothelial Progenitors as Cellular Vehicles for Oncolytic Adenovirus Anti-tumor Therapy" for submission to Cancer Research.

- Pilot project funding has been awarded through the UAB Breast Cancer SPORE mechanism based on work supported by this award.

- The "Cell Vehicle Working Group" has been created at the UAB Division of Human Gene Therapy to advance discoveries using this type of vector delivery/targeting system. The Cell Vehicle Working Group meets monthly to discuss project development and currently includes 6 members, which are Jerry Blackwell (P.I.), David Curiel (Division Director), Yosuke Kawakami (Post doctoral fellow), Laurisa Pereboeva (Group leader), Hui Li (Researh Assistant), and Jill Nagle (Graduate Research Assistant).

Conclusions

Important advancements have been made in the development of a readily available source of human CD34+ EPCs, which was a significant problem in the early phases of the project. We can now produce CD34+ EPCs on a weekly basis and more often if needed. In addition to the herpesvirus vector system, we have also established a second live-viral vector system using Ad. A strong argument can be made for using the Ad vector rather than the herpesvirus vector system, which includes both considerations of production and downstream translational issues. A formal change to the Ad vector system is being prepared for the Grants Officer for consideration. Most importantly, the project is now entering into the final *in vivo* testing phase where the real utility of the CD34+ EPCs delivery system will be rigorously evaluated. Whereas this is the most challenging element of the project, it is also the most exciting.

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